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The theory of breastmilk flavor exposure impacting later dietary variety in children was examined. Dietary intakes of 2-3 year olds of overweight (BMI > or = 25) mothers were analyzed. Mothers reported how the child was fed for the first 3 months. Participants were classified as either fully formula-fed group (FF, n=63) or fully breastfed group (BF, n=123) (mixed-fed group was excluded). Two-day 24-hr dietary recalls were conducted. The number (variety) of different fruits and vegetables (F&V) was counted over 2 days. Reappearing foods were not counted again. Bivariate relationships of maternal BMI, income, education, age, race, and feeding group were analyzed with variety. Variables were then entered into regression models to predict fruit, vegetable, and total F&V variety.

In bivariate analysis, variety of fruit was significantly higher in the BF (FF 2.7 +/- 1.5, BF 3.3 +/- 1.5; P=0.02), while neither vegetable (FF 2.8 +/- 1.6; BF 2.4 +/- 1.6), nor total F&V (FF 5.5 +/- 2.4, BF 5.7 +/- 2.3), varieties were significantly different between groups. In multivariate analysis, BF was higher when women were educated (P=0.0005) and had a lower BMI (BF 31.2 +/- 4.8; FF 32.9 +/- 6.3 kg/m^2; P=0.04). Maternal BMI was a significant negative predictor of fruit variety, while education was a positive predictor of fruit variety. No variable predicted vegetable variety. Education and BMI also predicted total fruit and vegetable variety (P=0.04, P=0.03). Bivariate analysis showed that maternal education and BMI were the significant predictors of fruit

and vegetable intake. Results of this study do not support the theory that exposure to flavors transmitted in breastmilk within the first 3 m of life affect later F&V variety.

RELATIONSHIP OF EARLY INFANT FEEDING (BREAST VS. FORMULA) AND FRUIT AND VEGETABLE VARIETY IN DIETARY INTAKES OF 2-3 YEAR OLDS, AS MAY PREDICT CHILD'S BMI Z-SCORE

by

LaCrystal A. Strong

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Approved by

_____
Committee Chair

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair _____

Committee Members _____

_____

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Date of Acceptance by Committee

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Date of Final Oral Examination

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CHAPTER I

INTRODUCTION

The yoke of chronic diseases in the world is becoming heavier and more costly to bear. It has been estimated that, in 2001, chronic diseases were the culprit in 60% of the 56.5 million worldwide reported deaths, and make up 46% of the global burden of disease (WHO 2002). These statistics are disturbing and expected to grow. By 2020, it is estimated that the overall burden of chronic diseases will balloon to 57%. It is estimated that by 2020, 3/4 of all worldwide deaths will be due to chronic diseases (WHO 1998). Of these chronic diseases, cardiovascular disease is the primary problem, perturbed by increasing trends of obesity and diabetes, both of which are presenting earlier in life (WHO 2003).

Amidst these alarming health statistics, it has been established that diets rich in fruits and vegetables are associated with health benefits such as reduced incidence of obesity, cardiovascular disease (Genkinger 2004, He 2006, He 2004, Hung 2004) and cancer in adults (Joshipura 2001, Hung 2004, Ziegler 1986). The World Health Organization recommends a total fruit and vegetable intake of $\geq$ 400 grams per day, or approximately 5 cups/servings per day (WHO 2003). Despite these known health benefits, adults are not meeting the recommendation for fruit and vegetable intakes (Casagrande 2007). Equally importantly, children are missing the mark as well

(Guenther 2006). Specifically, preschoolers aged 2-5 years often have inadequate intakes of fruits and vegetables (Dennison 1998). This issue is of concern because toddlers are in a critical growth stage and are also developing a palette for flavors and textures as novel foods are introduced and recur within the diet (Nicklaus 2009, Stang 2006). In addition, studies show that early eating habits not only affect weight in the toddler years, but can track into adulthood (Nicklaus 2009). Poor childhood diets can lay a foundation for adult disease later in life, as major biological and behavioral risk factors emerge in early life and persist to negatively impact health status through the life course (Aboderin 2002, WHO 2003, Barker 1989, 1993, 1995, Whincup 2002, Klesges 1995, Moller 1994).

Breastfeeding has been suggested as a strategy that may help increase novel food acceptance in infants, which may help address the problem of inadequate fruit and vegetable intakes in toddlers. Research suggests that breastfeeding may confer the benefit of flavor imprinting in the infant through exposure to a wider range of flavors via breastmilk than would occur in fully formula-fed infants. Research in this area has led to an endorsed policy statement regarding Dietary Recommendations for Children and Adolescents, by the American Academy of Pediatrics (AAP) suggesting that flavor exposures to the fetus and infant, via amniotic fluid and breastmilk, respectively, could influence "taste preferences and food choices after weaning (Gidding 2006)."

Presently, the American Academy of Pediatrics (AAP) recommends full breastfeeding for at least the first six months of life, citing increased novel food acceptance at weaning as a benefit of breastfeeding (AAP 1997). In addition, the American Heart Association suggests that the exposure to flavors transmitted through

breastmilk may impact later food choices, making breastfeeding an early strategy in promoting fruit and vegetable intakes in children (Gidding 2006).

Studies have evaluated predictors of fruit and vegetable consumption in toddlers and children. Results indicate that familial factors, such as parental control over feeding, parental intake of fruits and vegetables, mother's attitudes and health beliefs, and perceived home availability and access to fruits and vegetables were significant predictors of fruit and vegetable intakes (Zeinstra 2009, Wardle, 2005, Gibson 1998, Cooke 2003, Reinaerts 2007, Koui 2008). Choice, parental pressure to consume vegetables, and older age (7-8 years old or 11-12 years old) were positive predictors of vegetable intake, while using distraction to encourage consumption, a negative atmosphere, and positive information about vegetables were negative predictors of children's vegetable intake (Cooke 2003). Additionally, maternal education was the primary socioeconomic factor that predicted fruit and vegetable intake in children. Socioeconomic factors seem to contribute to the breastfeeding behavior of mothers as well as the fruit and vegetable intakes in children (Reinaerts 2007).

The majority of studies have evaluated fruit and vegetable consumption, as opposed to variety of fruits and vegetables consumed by preschoolers. The research is even scarcer among children who are born to overweight and obese mothers, and are therefore at risk for overweight or overweight, themselves. It remains to be seen how enduring an effect the flavor exposure during breastfeeding can have upon fruit and vegetable variety in at-risk-for-overweight and overweight children. The predictive strength of breastfeeding needs to be pinpointed in order to inform effective interventions

to help increase the fruit and vegetable intakes of preschoolers, and may represent an area of intervention in future research.

Specific Aim

The three-fold purpose of this study is to characterize the fruit and vegetable intakes of 2-3 year-olds, and to determine whether breastfeeding for the first three months of life is a significant predictor of fruit and vegetable variety at 2-3 years of age.

Research Questions

What fruit and vegetables are 2-3 year-olds, born to overweight mothers, consuming within each infant feeding category- fully formula-fed and fully breastfed for the first 3 months of life (BF)?

What are the major predictors of fruit, vegetable, and total fruit and vegetable variety in 2-3 year olds who were fully breastfed for 3 months versus those children who were formula fed?

What are the major predictors of child's BMI z-score in 2-3 year olds who were fully breastfed for 3 months versus those children who were formula fed?

CHAPTER II

REVIEW OF LITERATURE

Introduction

It has been well established that diet and nutrition are important modifiable factors in terms of promoting health through the life course (WHO 2003). Diets rich in fruits and vegetables are associated with health benefits such as reduced incidence of obesity, cardiovascular disease (Genkinger 2004, He 2006, He 2004, Hung 2004) and cancer in adults (Joshipura 2001, Hung 2004, Ziegler 1986). However, children are not meeting the recommendation for fruit and vegetable intake (Guenther 2006, Dennison 1998).

This fruit and vegetable intake deficit is a concern because toddlers are in a critical growth stage and are also developing a palette for flavors and textures as novel foods are introduced and recur within the diet (Nicklaus 2009, Stang 2006). In addition, the World Health Organization states that "major biological and behavioral risk factors emerge and act in early life, and continue to have a negative impact throughout the life course" (WHO 2003). Studies show that early eating habits not only affect weight in the toddler years, but can track into adulthood (Nicklaus 2009). Poor childhood diets can lay a foundation for adult disease later in life, as major biological and behavioral risk factors emerge in early life and persist to negatively impact health status through the life course

(Aboderin 2002, WHO 2003, Barker 1989, 1993, 1995, Whincup 2002, Klesges 1995, Moller 1994).

Therefore, the farther "upstream" that feeding behavior can be positively impacted in individual 2-3 year olds, the greater the health-promoting effects that should be seen amongst the population. This makes breastfeeding an early strategy in promoting healthy diets, rich in diverse fruits and vegetables, amongst preschoolers. The American Heart Association, with the support of the American Academy of Pediatrics, has stated that the infant's exposure to flavors transmitted in breastmilk, as opposed to limited flavor exposure during formula-feeding, may promote greater acceptance of novel flavors in solid foods at weaning, which may translate to a more varied diet later in life (Gidding 2006).

Some studies investigating the theory of increased food acceptance with breastfeeding exposure seem to support the theory. However, the endurance of this breastfeeding effect upon novel food acceptance into the toddler years, as well as the translation of flavor exposure during breastfeeding to increased fruit and vegetable variety at 2-3 years old remains to be seen. In evaluating the fruit and vegetable variety of preschoolers, it is useful to first describe the current status of preschoolers' diets, and then establish the scientific basis for this theory of impact upon dietary variety of flavor transmission during breastfeeding, followed by examining the predictors of fruit and vegetable variety and BMI z-score in 2-3 year olds.

Fruit and Vegetable Consumption in Preschoolers

The most updated, comprehensive evaluation of infant and toddler diets is the 2002 Feeding Infants and Toddlers study (FITS), sponsored by Gerber Products Company. The study population included infants and toddlers, age 4-24 months, and compared Hispanic and Non Hispanic infants and toddlers. This study is unique because of the validated 24-hour dietary recall evaluation technique and the multitude of in-depth nutrient analysis, food group analysis, portion size analysis, and macro- and micronutrient distributions that could be characterized according to ethnic differences that shape the United States (Hispanic or Non Hispanic).

Though the FITS study does not include toddlers older than 24 months, the study provides insight into the sources of energy and nutrients in infants and toddler diets, average portions of foods and type of foods commonly eaten by infants and toddlers (Fox MK, Reidy K, Novak T et al 2006, Fox MK, Reidy K, Karwe V et al 2006, Mennella 2006). Twenty-four-hour dietary recall data from 3,022 infants and toddlers from the United States were analyzed. 3,586 unique foods were reported in the 24-hours dietary recalls, and were then grouped into 71 groups. The nutrient content by weight of the food items were summed and divided across the study population to determine a percentage contribution for all 71 food groups. Among toddlers 12-24 months, juices were the second-ranking sources of total energy, contributing 6.4% of total calories, followed by sweetened beverages at 4.7% of total calories. These results show that fruit consumption in the way of juices, not whole fruits/canned/frozen fruits, is significant amongst toddlers (Fox MK, Reidy K, Novak T et al 2006).

Additionally, the FITS study gives insight into the average portions of fruits and vegetables consumed by toddlers, aged 19-24 months (Fox MK, Reidy K, Karwe V 2006). Foods that were consumed by at least 5% of the 3,022 infants and toddlers were included in the portion size averages. Portion sizes per eating occasion were calculated for each food and food group. An eating occasion was defined as the number of times the child was reported to have eaten or drank anything besides water and/or supplements. In terms or fruit and vegetable intake amongst 19-24 month-olds, the average portion size per eating occasion of commonly consumed fruits and fruit juices (mean±SEM) were: all fruits 0.6± 0.003 cups; canned fruit: 0.04±0.04 cups; all fresh fruit: 0.6±0.03 cups; fresh apple: 0.6±0.11 medium apple; fresh banana: 0.7±0.04 medium banana; fresh grapes: 0.3±0.02 cups; 100% Juice: 5.1±0.18 fl oz. The average portion size per eating occasion of commonly consumed vegetables (mean±SEM) were: All vegetables: 0.4±0.02 cups; Cooked Vegetables excluding french fries: 0.3±0.02 cups; Deep-Yellow Vegetables: 0.3±0.05 cups; Corn: 0.2±0.03 cups; Peas: 0.2±0.02 cups; Green Beans: 0.3±0.03 cups; Mashed Potatoes: 0.3±0.05 cups; Baked, and French Fries: 0.6±0.05 cups. In this group of subjects, the reported portion sizes for fruits and vegetables exceeded the Child and Adult Care Food Program (CACFP) portion size recommendations. However, it should be noted that, though 19-24 month-olds' intake fruit and vegetable intakes exceed CACFP recommendations for menu planning for 1-2 year-olds, these recommendations are the minimum established requirements. According to MyPyramid.gov, a 2-year old who is active at least 60 minutes per day needs one to one and a half cups of fruit per day and one to one and a half cups of vegetables per day. Following the minimum CACFP

guidelines would mean that only 1.5 servings of fruits and vegetables would be served to a 2-year-old in one day, which would not meet the MyPyramid recommendations. Standards such as the Dietary Guidelines for Americans, and MyPyramid are designed to speak to negative dietary habits that are emerging at younger ages (Fox MK, Reidy K, Karwe V 2006), but the CACFP recommendations are not.

The FITS study also evaluated the types of foods fed to infants and toddlers on a given day from the 24-hour dietary recall information (Mennella 2006). The top fruits consumed by 12-24 month old Hispanic toddlers were bananas (41.5%), apples (25.7%), berries (8.5%), melons (7.6%), and pears (7.3%). The top fruits consumed by 12-24 month old Non-Hispanic toddlers were bananas (30.9%), apples (22.0%), grapes (12.3%), peaches (9.6%), and berries (8.7%). The top vegetables consumed by 12-24 month old Hispanic toddlers were potatoes (43.5%), tomatoes (23.1%), carrots (18.6%), onions (11.8%), and corn (10.2%). The top vegetables consumed by their Non-Hispanic counterparts were potatoes (39%), green beans (19.6%), peas (12.8%), carrots (12.3%), and tomatoes (11.9%). This analysis shows that the top two fruits consumed were the same for Hispanic and Non-Hispanic toddlers- bananas and apples. From this study, it is evident that starchy vegetables are the most frequently consumed among Non Hispanic toddlers, while the Hispanic toddlers' vegetable intakes include more non-starchy vegetables.

Fetal Environment and Breastfeeding Exposure Theory

Foundationally, key research that first began to link fetal environmental exposure to odors and flavors was conducted by Mennella et al (Mennella 1995) who determined

that garlic ingestion by pregnant women created a detectable garlic odor in amniotic fluid. An adult sensory panel judging the odor of amniotic fluid samples from 10 women undergoing amniocentesis procedure, half of which consumed garlic essential oil capsules and the other half consuming placebo capsules, revealed that the odor of garlic was stronger in the amniotic fluid of 4 of the 5 women who had consumed the garlic essential oil capsule, versus those women who received the placebo capsule. Therefore, garlic ingestion by pregnant women significantly alters the odor of their amniotic fluid, raising further questions about infant exposure to additional substances through breastmilk and potential for an impact on breastfeeding.

Addressing this question is further research by Mennella and Beauchamp (Mennella 1993) who studied whether the breastfeeding behavior of infants would be modified due to repeated exposure to garlic via the mother's breastmilk. The study design entails three groups of mothers, who either consumed placebo pills or garlic essential oil capsules at one of two varied time points- at day 5-7 or day 8-10- to test infant response to amount and recency of garlic exposure. Two 4-hour test sessions were conducted, once at day 4, when placebo capsules were administered, and then once at day 11 when all mothers consumed an essential oil garlic capsule. Infants were weighed before and after feeding to obtain breastmilk intake volumes. Between the baseline test session, and the endpoint test session, researchers concluded that infants who had no exposure to garlic during the experimental period, but experienced garlic volatiles for the first time during the endpoint test session had a significantly higher intake of breastmilk, and nursed longer than infants who had prior exposure to garlic during the experimental

period and were re-exposed to garlic during the endpoint test session. Overall, infants stayed at the breast longer at the endpoint test session when all mothers had consumed the garlic capsules, versus the day-4 test session when all mothers received placebo capsules. These results show that flavors in breastmilk are detectable by the infant and can have an impact upon feeding behavior, and that prior experience to a substance can also affect subsequent feedings. This research raised other questions and opened the way for further research to be conducted regarding novel flavor acceptance, particularly to solid foods at weaning.

Gerrish and Mennella (Gerrish 2001) tested the hypothesis that acceptance of novel foods by formula-fed infants (n=48) could be influenced by incorporating a variety of flavors when solid foods are first introduced. A twelve-day study was conducted, during which, 3 groups of infants (n=16 each) belonged to a carrot group, a potato group, and a variety group. All infants were exposed to carrots on the first day, and then, were either randomized to a feeding group- carrots daily, potatoes daily, or a variety (peas, potatoes, squash) daily (days 2-10). On the 11^{th} day of the study, each group was exposed to carrots, and then on day 12, a novel food (puréed chicken) was introduced. Results showed that only infants consuming either carrots or a variety of vegetables ate significantly more carrots (the target vegetable), whereas, only infants who consumed a variety of vegetables had better acceptance of the novel food. From this study, it is clear that variety during early weaning has an impact upon novel food acceptance, but the type of feeding, either breastfeeding, mixed-feeding, or formula-feeding was not a factor in this experiment.

Expounding upon these results, Mennella and Beauchamp (Mennella 2002) explored the relationship between varying formula types fed in infancy (milk-based, protein hydrolysate and soy) and flavor preferences in 4- to 5-year old children. Results indicated that, compared to children fed milk-based formula, children fed protein hydrolysate formulas (n=50) were more likely to prefer sour-flavored juices. More notably, mothers' reports indicated that children fed protein hydrolysate or soy formulas (more sour formulas) were significantly more likely to prefer broccoli versus those children who were fed milk formulas. Therefore, the previous two studies, taken together reveals how formula-fed infants are influenced by variety of flavor exposure- whether it occurs by the type of formula or the type of fruit/vegetable repeatedly offered to an infant. This begs the question of whether breastfed infants experience the same results.

Continuing the thought of early infant exposure, this time, comparing the effects of breastfeeding and dietary experiences on acceptance of a fruit and a green vegetable by 4-to 8-month-old infants, Forestell and Mennella (Forestell 2007) assigned 45 infants, 44% of whom were breastfed, to one of two treatment groups. One group was fed green beans, while the other was fed both green beans and peaches at the same time for 8 days. Results showed that for both breastfed and formula-fed infants, repeated exposure to green beans, with or without peaches, yielded greater green bean consumption. While these results concerning consumption of fruits and vegetables in infancy were not significant, there still remains the question about infancy exposure to flavors in breast milk and dietary variety in preschoolers (Forestell 2007).

An additional study by Mennella et al further evaluated flavor imprinting and its potential impact upon novel food acceptance (Mennella 2008). In a 12-day experimental study, 74 mother-infant dyads in a nested Vegetable Exposure Study were randomized to a Repeated Exposure, Between-Meal (BM) Variety, or BM-Within-Meal (BM-WM) Variety group. Researchers aimed to investigate the effects of variety-- Repeated Exposure of one vegetable or several vegetables-- and timing-- WM-, BM-, or WM-BM-variety. Days 1 and 2 were conducted on-site, where researchers measured acceptance of the target food. After an 8-day home exposure, where mothers fed their infants according to variety and timing assignments, dyads returned to the research site on days 11 and 12 for reassessment of target food acceptance. Researchers quantified food acceptance by total caloric intakes, length and rate of feeding and mothers' rating of infant's enjoyment of food after exposure. There was an increase in intake (Mean (SD) = green beans: 3.2 (0.6) times more; carrots: 3.6 (1.7) times more; spinach: 5.1 (2.2) times more; ($P < 0.05$) in BM-WM group. The BM-WM group started Mean (SD) = 8.7 (2.0) kcal spinach intake, but after experiencing spinach twice during the 8-day home exposure, intake increased to Mean (SD) = 19.9 (4.2) kcal. Equal exposure in the BM Group was nonsignificant. These findings reveal that sensory differences of the BM-WM group may have accelerated food acceptance. The authors concluded that infants are able to discern different flavors, but opportunities to experience the food and variety promote food acceptance. Strengths of the study include food record and phone contact compliance measures during home-exposure and an acclimation period of infants to study materials, which reduced confounding effects. The study was limited because the experiment was

not targeted at clarifying differences in food acceptance in BF versus FF infants, so the questionnaire assessing BF practices was not validated. Also, eligibility criteria requiring minimal food exposure may have been too loose such that infant history with foods may have confounded results. Nonetheless, this study shows that varied and repeated exposure to flavors promotes greater subsequent intake of the novel food item as well as increased willingness to consume new foods.

Research by Maier et al, also observed the interaction between BF experience and early variety in weaning and infants' novel food acceptance, but did so in greater detail than previous studies (Maier 2008). The study involves the longest exposure period and tested the most levels of variety. A multi-phase, two-site randomized, controlled trial assessed 143 mother-infant dyads who completed 3 phases: A) vegetable introduction and test of first new vegetable; B) repeated, alternating exposure to carrots and first new test food (Zucchini-Tomato (ZT)), and test of second new vegetable (peas); C) all groups-alternating novel meat and vegetable and test of novel fish acceptance. During phases A and B, infants were randomized to experience no variety, low, or high variety during home exposure. ANOVA was used to determine that both BF and level of variety were individually and collectively associated with increased intake of new food ($P < 0.0001$), with the greatest increase in new food intake occurring in the highest variety group. Changes in time between phases A and C showed a significant interaction between BF experience and food ($P = 0.02$), with a greater intake difference in ZT and pea intakes in BF versus FF infants. The authors concluded that a significant interaction between BF and food variety exists, affecting novel food acceptance. The study's methodology was

well-designed. The study coincided with the infants' first solid-food exposure, eliminating any interaction with their past experience with solid food. The extended exposure period allowed more time for development of flavor acceptance, while the gradations in variety allowed for a more sensitive assessment of the influence of variety. Though researchers did not further separate exclusive breast-feeding from breast-feeding, this study solidifies the hypothesis that BF is involved in flavor programming of infants, and contributes a novel approach to looking at weaning, by introducing the additional stage of weaning to meats (Maier 2008).

Continuing with this thought, Noble and Emmett conducted a one-day cross-sectional evaluation of dietary intake of BF and FF infants in weaning, which connects the idea of flavor programming (Noble and Emmett 2006). Uniquely, this study quantified solid food, formula, and breast-milk intake during weaning. This is one of few studies to capture intake during weaning, rather than after weaning. Researchers found that, at weaning, FF infants were less likely to consume fruits and vegetables and more likely to consume commercial infant drinks. Authors postulated that this difference in consumption patterns at weaning may contribute to later growth differences in FF versus BF infants. While reliability of the data is limited due to the brevity of the study, it contributes a starting point to experimentally investigate weaning practices of BF and FF infants. Yet, questions remain about the longevity of the increased novel food acceptance and whether this initial increased novel food acceptance will translate to increased dietary variety in preschoolers (Noble and Emmett 2006).

Skinner et al were the first to take a longer term approach by investigating food-related experiences, particularly breastfeeding duration, as a predictor of dietary variety in school aged children (Skinner 2002). Essentially, Skinner et al combined the premises of all the aforementioned studies, by using the notions that infants are more accepting of those flavors they received in breast milk transmission; similarly to formula-fed infants in the Gerrish et al study (Gerrish 2001), breastfed infants will have increased receptivity of novel foods based on dietary variety; and the idea of repeated exposure to potentially increase dietary variety, as tested by Forestell and Mennella (Forestell 2007). Skinner et al interviewed child/mother dyads (n=70) 7-8 times when the child was 2-24 months of age, and then again when the child was 6, 7, and 8 years of age. The following variables were analyzed: breastfeeding duration, age of infant at vegetable/fruit introduction, mother's preference using Total Diet Study, Early Vegetable Variety–(# different vegetables), Early Fruit Variety–(# different fruits), Early Vegetable Exposure –(# of times vegetable appeared in recall), and Early Fruit Exposure–(# of times fruits appeared in recall). Results indicated that breastfeeding duration was shown to be a positive indicator of fruit variety in older children (P=0.0172). Also, vegetable variety was only predicted by mother's vegetable preferences ($R^2 = 0.085$). It is important to note, that, in both instances, the models are fairly weak, indicating other contributing factors in the results that are unaccounted for in the study. In addition, the only factor about the mother that was assessed was the mother's preferences for vegetables, assessed when the child was 28-36 months and 8 years old. The study population was homogenous, in that, all participants were white and of a high socioeconomic class. Therefore, these variables

were not including in the prediction modeling of fruit and vegetable variety. While this study contributes to the knowledge base regarding dietary variety in children, as predicted by breastfeeding exposure, this study was conducted among school-age children, aged 6-8 years, which does not include the vital preschool developmental stage of children, aged 2-5 years. In addition, this study population is not representative of the United States. This study does not address the relationship of mother's weight status, child's weight status, education, nor income on fruit and vegetable variety (Skinner 2002).

Predictors of Fruit and Vegetable Consumption in Preschoolers

Predictors of fruit and vegetable consumption by children have been evaluated by across a wide range of variables- familial, psychosocial, behavioral, knowledge, attitudes, and beliefs (Zeinstra 2009, Wardle 2005, Reinaerts 2007, Gibson 1998, and Koui 2008). Zeinstra et al investigated several child-feeding strategies from the parent and child perspective using an age-appropriate questionnaire. Two hundred forty two Dutch children, ages 4-5 years, 7-8 years, and 11-12 years and their parents were included in the study. Parents answered survey questions that assessed their own eating practices, and also completed questions of the Child Feeding Questionnaire pertaining to parental control measures, termed—monitoring, restriction, and pressure to eat. Children also completed a photograph questionnaire to assess food preferences using a scale of 1-5 for rating. The child food preferences survey items were grouped into the following categories: fruit, vegetable, fruit juices, neutral products and energy dense products. Researchers used factor analysis to identify parental child-feeding strategies that may

exist between and amongst fruits and vegetables in their children. Six significant factors identified in child-feeding strategies for vegetables were: positive information, distraction, choice, negative atmosphere, pressure, and taste masking, each $P < 0.001$. Four significant parental child-feeding strategies for fruits were identified as follows: negative atmosphere and pressure, positive information, choice, and availability, each $P < 0.001$. Regression was then used to relate these factors to the effect they may have upon fruit and vegetable intake in the child.

The key finding from this study by Zeinstra et al was that parents used different strategies in feeding their children vegetables versus fruits and that the atmosphere of vegetable feeding was more negative than that related with fruit feeding. The regression model of children's vegetable intake prediction explained 47% of the variance in children's vegetable intake. Parental child-feeding strategies of distraction, negative atmosphere, and positive information were negative predictors of vegetable intake, while parental vegetable intake, choice, pressure, child's older age (7-8 and 11-12 year olds) turned out to be positive predictors of vegetable intake.

The regression model of children's fruit intake accounted for 28% of the variance in children's intake. Parental intake and choice emerged as significant positive predictors of fruit intake. Negative atmosphere was also a negative predictor of fruit intake, as seen with vegetable intake. However, an important difference to note is that pressure was a negative predictor of fruit intake in children, despite it being a positive predictor of vegetable.

Zeinstra et al also assessed children's liking of different groups of fruits and vegetables. Children's rating showed that they liked vegetables the least (mean = 3.1±0.9. Neutral foods such as potatoes, milk, and bread and the most neutral rating by the children (mean = 3.8±0.8). Preference for fruit and fruit juices were second-most liked grouping (mean = 4.0 ±1.0). Finally, energy-dense food (i.e.- chocolate and french fries) were the most liked among children mean = 4.6±0.6). These results suggest that vegetables not simply not well-liked by children, who instead seem to prefer sweet-tasting fruit and fruit juices, along with more energy-dense, satiating foods. In addition, these results also support the findings of prior research that vegetable intake more be more difficult to promote (Mennella 2008).

Furthermore, Wardle et al investigated the relationship between parental control in child-feeding strategies (Restriction- restricted access to high-fat, sweet foods; and Pressure to eat- reinforcement to spur the child to eat more fruits and vegetables) and children's fruit and vegetable intake. Wardle and colleagues found an inverse relationship between parental control and less frequent fruit and vegetable consumption by the child ($r = -0.17$). Also, parents who exerted more parental control measures also reported eating less fruits and vegetables ($r = -0.16$) and also tended to have more neophobic children ($r = 0.26$). Researchers found parental fruit and vegetable intake to be a significant predictor of child fruit and vegetable intake via survey of parents of 564 2-6 year olds in London, England. In this study, researchers also assessed neophobia in children using the Child Food Neophobia Scale, and found it to be a significant predictor of both fruit and vegetable consumption. Both of these significant predictors of fruit and

vegetable intake in children were significantly related to the control that parents used regarding children's intake. However, after adjusting for both child neophobia and parental intake, parental control did not retain significance.

Additional research by Gibson et al, Reinaerts et al, and Koui et al reinforces the importance of an environment conducive to increased fruit and vegetable intake in children (Gibson 1998, Reinaerts 2007, Koui 2009). Gibson et al showed that the mother's health beliefs about the importance of fruits and vegetables in disease prevention was a predictor of vegetable intake in children aged 9-11 years. Similar to other research, the mother's own intake of fruits and vegetables was predictive of the fruit and vegetable intake in the child (Gibson 1998).

In a group of 4-12 year olds, Reinaerts et al (Reinaerts 2007) showed that the strongest predictor of fruit and vegetable consumption was habit, even amongst other variables such as availability, exposure, and parental consumption. This study also showed different behaviors in fruit and vegetable consumption, aligning with the findings by Zeinstra et al (Zeinstra 2009).

Koui et al also evaluated home availability of fruits and vegetables and its relationship with intake by children. In a sample of 167 students in fifth and sixth graders enrolled in one of five primary schools in Pyrgos, Greece, who completed Food Frequency Questionnaires, home availability of fruits and vegetables was associated with increased fruit and vegetable consumption in the children (Koui 2008).

Lastly, Cooke et al evaluated demographic, familial, and trait predictors of fruit and vegetable consumption by 2-6 year-old preschool children and found that, after

controlling for parental intake of fruits and vegetables and child food neophobia, breastfeeding impacted fruit consumption but not vegetable consumption. Vegetable consumption was related only to the child's gender and the child's enjoyment of food. Maternal education was also positively related to the child's vegetable intake. These results represent an interplay between demographic, familial and traits of the child that influence fruit and vegetable intake (Cooke 2004).

Conclusion

Past research seems to indicate that there is a firm foundation for the theory that breastfeeding exposure early in life can influence fruit and vegetable variety. However, it remains unclear how enduring an effect breastfeeding flavor exposure can have upon fruit and vegetable variety. Additionally, the FITS study identified the most commonly consumed fruits and vegetables in toddlers. However, the most commonly consumed vegetables in children at risk for overweight or overweight have not been identified. The itemized list of which fruits and vegetable 2-3 years are consuming has not yet been presented. Prior studies that examined child intake have grouped the fruit and vegetables by class (i.e. - dark yellow; dark green). Predictors of fruit and vegetable intakes appear to be demographic variables such as mother's education, child's age, and gender and by traits such as child's enjoyment of food and food neophobia.

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CHAPTER III

PREDICTING FRUIT AND VEGETABLE VARIETY AND BMI Z-SCORES OF 2-3 YEAR OLDS BORN TO OVERWEIGHT AND OBESE WOMEN

Introduction

Preschoolers aged 2-5 years often have inadequate intakes of fruits and vegetables (Dennison 1998). This issue is of concern because toddlers are in a critical growth stage and are also developing a palette for flavors and textures as novel foods are introduced and recur within the diet. In addition, studies show that early eating habits not only affect weight in the toddler years, but can continue into adulthood (Nicklaus 2009). Poor childhood diets can lay a foundation for adult disease later in life, as major biological and behavioral risk factors emerge in early life and persist to negatively impact health status through the life course (Aboderin 2002, WHO 2003, Barker 1989, 1993, 1995, Whincup 2002, Klesges 1995, Moller 1994).

Therefore, the farther "upstream" that feeding behavior can be positively impacted in individual 2-3 year olds, the greater the health-promoting effects that should be seen amongst the population. This makes breastfeeding an early strategy in promoting healthy diets, rich in diverse fruits and vegetables, amongst preschoolers. The American Heart Association, with the support of the American Academy of Pediatrics, has stated that the infant's exposure to flavors transmitted in breastmilk, as opposed to limited flavor exposure during formula-feeding, may promote greater acceptance of novel flavors

in solid foods at weaning, which may translate to a more varied diet later in life (Gidding 2006).

Presently, the American Academy of Pediatrics (AAP) recommends full breastfeeding for at least the first six months of life, citing increased novel food acceptance at weaning as a benefit of breastfeeding (AAP 1997). In addition, the American Heart Association suggests that the exposure to flavors transmitted through breastmilk may impact later food choices, making breastfeeding an early strategy in promoting fruit and vegetable intakes in children (Gidding 2006).

However, there is a gap in the literature regarding whether the initial benefits of flavor exposure via transmission through breastmilk can confer a sustained impact on fruit and vegetable intakes. Mennella et al (Mennella 2008) showed that breastfeeding, as opposed to formula-feeding, made a significant difference in the acceptance of new foods when infants were exposed to highly varied fruits and vegetables in the diet. Skinner et al (Skinner 2001) also found that breastfeeding was a significant predictive factor of fruit and vegetable variety, but of older school-age children, six, seven, and eight years old. However, no prior studies have investigated the relationship between breastfeeding exposure and fruit and vegetable variety of preschoolers, aged 2-3 years.

In addition to early infant feeding, other factors such as income, education, race, and maternal BMI may influence fruit and vegetable intake among children (Koui 2008). Therefore, the objective of this study was to describe the variety of fruit and vegetable intake of 2-3 yr olds with overweight or obese mothers and to determine if breastfeeding or formula feeding was related to fruit and vegetable variety at 2-3 years of age. A

second objective was to determine if fruit and vegetable variety was associated with the child's BMI z-score. The theoretical model is shown in Figure 1.

Methods

Participants

Participants for this study were recruited from two studies—the Kids and Adults-Now Defeat Obesity (KAN-DO) study and the Active Mothers Postpartum Too For Twos! Study (AMP-Too) (Østbye 2004).

Kids And Adults-Now Defeat Obesity (KAN-DO) study

The KAN-DO study is a two-site (University of North Carolina Greensboro and Duke University), randomized, controlled family-based intervention to promote healthy families. This study examines whether teamwork between mother and child in the areas of healthy eating, physical activity, and reducing sedentary behaviors, in addition to instruction on parenting skills and behavior change for the mother, are helpful in promoting maintenance of a healthy weight in normal weight children and relative weight reduction in overweight children.

KAN-DO participants were recruited by a postcard mailing to their home address. Home addresses and phone information of women in the Triad (Greensboro, Winston Salem, High Point) and Triangle (Raleigh, Durham, Chapel Hill) regions who had recently applied for a birth certificate were obtained by a mailing list from the Department of Vital Statistics. (See Appendix A for Recruitment Postcard). In addition, brochures and posters were displayed in Triad and Triangle OB/GYN and pediatric practices and health departments and within the greater community (libraries, local

stores, and community bulletin boards). In all instances, a toll-free phone number was listed on the recruitment tool for potential participants to call, if interested.

At three-six months postpartum, the mothers were contacted by trained research personnel, using the phone information provided from the Department of Vital Statistics. Research personnel screened the participants for eligibility based on the following criteria: recent delivery of a baby, a preschooler in the home (2-5 years old), and a current BMI $\geq$ 25 (confirmed by measurement at baseline visit for determination of final inclusion). Women were also asked if they had any medical conditions that would exclude them from the study, such as the physical inability to walk one mile or if the child had a physical condition that prevents him or her from walking one mile.

Interested and eligible mother/child dyads were then scheduled to attend a baseline data collection session at the most convenient study site, either at the Duke or UNCG. At this visit, after complete explanation and description of the study criteria, mothers were asked whether or not they would like to participate in the study. If the answer was affirmative, the mother signed a consent form (See Appendix B). All protocol was approved by the Institutional Review Boards of the University of North Carolina Greensboro and Duke University.

Active Mothers Postpartum TOO- For Twos! (AMP-TOO) study

The purpose of the AMP-TOO study was to examine the dietary intakes of 2-year-olds born to overweight and obese mothers and to identify predictors of the child's BMI. Participants for the AMP TOO study were recruited from participants of the AMP

study. Participants were asked at their two-year postpartum AMP visit if they would like to participant in the AMP-TOO study.

To be eligible for the AMP-TOO study, the mother must have had a BMI $\geq$ 25 kg/m^2 prior to pregnancy, be English-speaking, at least 18 years of age, and be at least two months postpartum. Mothers signed consent forms (See Appendix C). All protocol was approved by the Institutional Review Boards of the University of North Carolina-Greensboro and Duke University.

Demographic Information

Participants in both the KAN-DO and AMP-TOO studies completed questionnaires. In both studies, demographic information regarding race, maternal education, and income was collected. KAN-DO participants were mailed a baseline survey (during the three to six month postpartum period). Mothers were encouraged to complete the questionnaire prior to the baseline visit. The questionnaire was reviewed for completion by a study staff member. AMP-TOO participants completed their questionnaire at 2 years postpartum.

Anthropometrics

Weight and height baseline measurements for the KAN-DO study were performed at three-six months postpartum and at two years postpartum for the AMP-TOO study. Heights and weights of both mothers and children were measured using a Seca portable stadiometer (Columbia, MD) and a Tanita BWB-800 scale (Arlington Heights, IL), respectively, with the participant wearing light clothing and no shoes. For both the KAN-DO and AMP-TOO studies, the measured preschooler heights and weights were used to

compute body mass index (BMI) values (body weight (kg)/height (m^2). Using the child's BMI and age, a BMI z-score was determined, based upon the WHO child growth curves (WHO 2006). A BMI between the 5^{th} and 84^{th} percentiles corresponds with a BMI z-score of ($0 < z\text{-score} < 1.036$), and is considered within the healthy range. A BMI z-score of ($1.036 < z\text{-score} < 1.645$) is considered at risk for overweight. A BMI z-score of ($z\text{-score} \geq 1.645$) is considered overweight.

After the completion of all baseline data collection, mother/child dyads were then randomized to a research group. All procedures for each study were approved by the University of North Carolina Greensboro and Duke University Institutional Review Boards.

Diet Assessment

Two 24-hour recall dietary recalls of the preschoolers were collected from their mother in both the KAN-DO and AMP-TOO studies using the Nutrient Data Systems for Research (NDS-R) software system (version 8.0 and 7.0, respectively, Minneapolis, MN). Prior to the interviews, those mothers indicating that their preschooler attended daycare, were given a Daycare Preschooler Intake Record (See Appendix D) to provide to the daycare personnel to record the items and amount consumed by the preschooler. Mothers referred to this record during the 24-hour recall. In addition, all mothers were provided a two-dimensional guide, representing food volumes and eating dish sizes. During the interview, mothers were asked what their preschooler consumed during the previous day. Interviewers guided the participants through estimating portion sizes by suggesting a specific page from the two-dimensional guide and had the participant choose

the dimensions closest the food item that she consumed. The multiple-pass method allowed the participant to think back through her previous day and recall all foods and beverages consumed with three guided reviews of all entries to encourage the participant to recall all items consumed.

Fruit and Vegetable Variety Count

Fruit variety, vegetable variety, and total fruit and vegetable variety were tallied for each participant using the NDS-R output spreadsheet, which listed each food reported for the preschooler. To count variety, each new fruit and vegetable appearance was counted for each participant over the 2-day period. All fruits and vegetables were allowed to count only once for a participant. Reappearing foods were not counted, only newly appearing fruits/vegetables. For example, if a toddler consumed bananas on two occasions during the two-day data collection period, "bananas" was counted as one toward the fruit count. One hundred percent fruit juices, regardless of the flavor, were counted only once. Fruit drinks containing less than 100% juice were not counted. The American Academy of Pediatrics recommends that no more than 6 fl. oz of 100% juice be consumed by 2-3 year olds per day (AAP 2001). Since intake of 100% juice, according to the recommendation by the American Academy of Pediatrics, is considered towards the fruit servings for preschoolers in MyPyramid, it was deemed appropriate to consider 100% juice toward the variety count in this analysis.

Furthermore, fried potatoes (e.g. - french fries, tater tots, and hash browns) were only counted as one vegetable. However, potato chips were not included. Tomatoes appearing in marinara sauce and cream of tomato soup were counted as one towards the

vegetable count. However, any tomato sauces that may have been used in pizza and lasagna fillings were not were included, because they are not counted as a vegetable in the NDS-R output.

Additionally, fruits and vegetables that may appear in different forms, such as twice baked potato and creamed potatoes were counted as only one towards the vegetable count. Foods that appear both raw and cooked/processed were counted as two items towards the variety count, such as a raw apple and applesauce and raw carrots and cooked carrots. Serving size was not taken into consideration for the fruit or vegetable to be counted, only the fact that the fruit or vegetable appeared in the dietary recall.

Infant Feeding History

Preschoolers' breastfeeding history information was collected from baseline questionnaires of both the AMP-TOO and KAN-DO studies. At baseline, or when the study-enrolled preschooler was 2-3 years old, KAN-DO mothers reported the preschooler's infant feeding history. AMP-TOO study participants reported their preschooler's infant feeding history during the AMP study, via questionnaire, at 12 months postpartum. Both studies asked the mothers to think back to the preschooler's first year of life and retrospectively report the mode of infant feeding for *each* month during the preschooler's first year. On the questionnaire, for each month listed (0-12 months), mothers checked the appropriate box for having breastfed only, combined formula and breastmilk, or formula-fed only for that particular month.

Infant Feeding Groups

Based upon the mother's month-by-month report of how she fed her preschooler for the first three months of life, the preschooler was then assigned to one of two groups: the fully formula-fed group (FF) or the group wherein which preschoolers were fully breastfed for at least three months (BF). Assignment to the fully formula-fed group indicated that the mother reported that she formula-fed for each month during the first three months of life, or that she reported having combined formula and breastmilk for only the first month of life, but continued with full formula feeding for months two and three. This allowance included the mothers who may have initiated breastfeeding at the infant's birth, but were unable to continue. Assignment to the three-month minimum fully breastfeeding group indicated that the mother reported that she fully breastfed the preschooler through the third month of life.

Assessing the mode of infant feeding for the first three months of life is important for the present research question because the introduction of solid foods would have occurred for the majority of preschoolers between four and six months. To eliminate the influence of introduction of solid foods on dietary variety, mode of infant feeding was assessed for only the first three months of life.

Statistical Analysis

Statistical analyses were performed using JMP software version 7.0 (SAS, Cary, NC). Chi-square and One-Way Analysis of Variance tests were used to compare characteristics between the formula-fed preschoolers and those fully breastfed for at least three months of life. Chi-square analysis was performed to define the relationships of

education, income, race, and toddler sex with feeding group. ANOVA was used to determine group differences in mothers' BMI, mothers' age, average daily fruit and vegetable servings, fruit variety, vegetable variety, and total fruit and vegetable variety. $P \leq 0.05$ was considered statistically significant for all analyses.

Bivariate relationships of fruit and vegetable variety and BMI z-score were assessed with the following variables: education, income, race, mother's age, mother's BMI, study in the dyads participated, and the mode of infant feeding (breastfed at least three months or formula-fed). The study in which the preschooler participated was included as a variable in order to adjust for the fact that the 24-hour dietary recall data were gathered at different time points in the two separate studies- one at the end of study (AMP-TOO) and the other at baseline (KANDO). $P \leq 0.05$ was considered statistically significant.

Generalized linear models were also constructed to evaluate predictors of fruit and vegetable variety and then to evaluate predictors of child's BMI z-score. A generalized linear model based upon a Poisson distribution was constructed to compare the strength of each predictor toward fruit variety, vegetable variety, and total fruit and vegetable variety. The following variables were evaluated in relationship with fruit variety, vegetable variety, and total fruit & vegetable variety: mother's education, income, age, race, BMI, study participation (AMP-TOO or KAN-DO), and preschooler's infant feeding group. For the following categorical variables- education, income, race, study classification, and infant feeding group- the following level was used, respectively, as the reference point in analysis: less than or equal to a high school degree, less than or

equal to $30,000 per year, white race, AMP-TOO study, and formula-fed. The continuous variables- mother's age and BMI- were evaluated in relation to the cohort averages: 31.48 years, and 31.81 kg/m^2, respectively. A Poisson distribution was used in this analysis because the outcome variables- fruit and vegetable variety- were counts performed on each preschooler, which created a discrete distribution, versus a continuous distribution. A normal distribution was used to model the predictors of child's BMI z-score, since BMI z-score is characterized by a continuous distribution. The aforementioned variables were also included in the BMI z-score prediction model.

Results

Participant Characteristics

The total numbers of participants from the KAN-DO and AMP-TOO studies were: FF = 63 preschoolers and BF = 123 preschoolers. The derivation of the final sample size from the two studies is shown in Figure 2.

Overall, participant characteristics between the formula-fed preschoolers and the preschoolers who were fully breastfed for the three months were similar. However, the breastfeeding mothers were significantly more highly educated (P=0.0005), more likely to be white (P=0.002), and tended to have a lower BMI (P=0.04), when compared to the mothers of the formula feeding group (Table 1).

Fruit and Vegetable Intake

Breastfed preschoolers had a significantly greater variety of fruits over a two-day period (P=0.02), as compared to those preschoolers who were formula-fed (Table 1).

However, there were no differences in variety of vegetables between groups. Differences in totaled fruit and vegetable variety intakes were also not significant between groups.

The ranges of both fruit and vegetable variety reported in this group of preschoolers were from 0-7 unique fruits reported, and 0-7 unique vegetables. One hundred percent juice, bananas, and apples were the top three most frequently reported fruits (Table 2) among both infant feeding groups. However, 100% fruit juice was reported more frequently for the formula-fed preschoolers, as compared to the breastfed preschoolers (74.6% versus 69.11%). Green beans, tomato sauce, and fried potatoes were among the top three most frequently reported vegetables between the formula-fed and breastfed preschoolers (Table 3).

Since consumption of dark green and orange vegetables is typically suboptimal in Americans, The United States Department of Agriculture's MyPyramid further stratifies the recommendations for total vegetable intake by making specific recommendations for dark green and orange vegetables (Guenther 2007). To address this caveat of variety, the percentage of dark green and orange vegetables that contributed to the total vegetable count was assessed in both infant feeding groups. Of the 25 distinct vegetables reported amongst the formula-fed group, 24%, or six items, were dark green/orange. The breastfed group had a higher percentage of variety coming from dark green/orange vegetables, at 30% (9 distinct vegetables) of the 30 total vegetables reported (P=0.01) (See Table 3).

In addition, the fruit variety was further stratified into total fruit and whole fruit. Of the reported fruits, applesauce, fruit leather, and 100% juice were excluded from the

whole fruit category. Breastfed preschoolers had a higher degree of variety from whole fruits (89.29%) than did the formula-fed preschoolers (83.33%) ($P < 0.01$) (See Table 2).

Predictors of Fruit and Vegetable Variety

Bivariate relationship analysis of each variable with two-day fruit variety showed that all variables, except the study in which subjects participated, were independently related to fruit variety. Maternal education, income, white race, mother's age were significantly positively associated with increased variety of fruits consumed. BMI held an inverse relationship with variety of fruits consumed. That is, having a higher education, being of a higher socioeconomic status, being white, and a lower BMI were all independently significantly related to having a preschooler who consumed a greater variety of fruits. The same relationships were not found with vegetable variety. In fact, there were no variables that were significantly associated with vegetable variety in bivariate relationship. However, the bivariate analysis data suggest that being of a non white race ($P=0.05$) may have a stronger relationship than infant feeding group with vegetable variety in this group of preschoolers. The bivariate relationship of totaled fruit and vegetable variety with each variable was similar to results seen fruit variety and each variable, except that KAN-DO study participants were more likely to have lower total fruit and vegetable variety, and income was no longer significantly related to variety.

After adjusting for all variables in one overall model of predictors of two-day fruit variety, mother's education, and BMI remained statistically significant ($P=0.04$) and ($P=0.02$) (See Table 4). Having attained a college degree or higher and having a lower

BMI increased the variety of the preschooler's two-day fruit intake. The mode of infant feeding was no longer significant when the other variables were included in the model.

As the lack of significance of any bivariate relationship with vegetable variety suggested, the overall generalized linear model of vegetable variety also revealed no significant predictive strength of any variable towards vegetable variety (Table 5).

Totaling the fruit and vegetable variety counts produced similar results as those found in the fruit variety prediction model. Both mother's education and BMI emerged as statistically significant predictors of two-day total fruit and vegetable variety, even after simultaneously evaluating all measured variables in the overall model (P=0.04 and P=0.03, respectively) (Table 6). Higher education was a significant, positive predictor of two-day fruit and vegetable variety, while the mother having a higher BMI was a significant, negative predictor two-day fruit and vegetable variety.

Predictors of Child's BMI Z-score

Bivariate analyses were conducted between child's BMI z-score and each of the following variables: maternal education, income, race, mother's age, mother's BMI, study in which the preschooler participated, the preschooler's infant feeding group, and the preschoolers two-day fruit and vegetable variety. Bivariate analysis results showed that maternal education, the difference in study enrollment, and both fruit variety and total fruit and vegetable variety were independently, significantly negatively associated with the child's BMI z-score. This means that the preschooler's mother having a higher education, the preschooler having participated in the AMP-TOO study, and the preschooler having a greater amount of both fruit variety and total fruit and vegetable

variety were all independently related to the child having a lower BMI z-score, or being closer to a healthy weight.

The generalized linear model of child's BMI z-score that included two-day fruit variety showed that both maternal education (P = 0.01) and fruit and vegetable variety (P = 0.02) were significant predictors of the child's BMI z-score. This means that the *most* overweight women also had preschoolers who had the lowest reported variety of fruits and vegetables.

Next, the generalized linear model of child's BMI z-score that included two-day vegetable variety showed only maternal education to be a strongly significant predictor of the child's BMI z-score (P = 0.006). The same model using totaled fruit and vegetable variety to the generalized linear model of child's BMI z-score showed that maternal education and total fruit and vegetable variety were both significant negative predictors of the child's BMI z-score, (P = 0.01 and P = 0.04, respectively) (See Table 7).

Discussion

In this study population of 2-3 year-olds, born to overweight mothers, those infants who were fully breastfed for the first three months consumed a significantly greater variety of fruits, but not vegetables, than those who were formula-fed. However, when controlling for maternal factors, such as mother's education and BMI, this was no longer significant. Since all of the mothers involved in the AMP-TOO and KAN-DO studies were either overweight or obese, as required by study inclusion criteria, and BMI still emerged as a significant predictor of variety, this means that mothers who were on the higher end of the spectrum of BMI, or the most overweight, tended to have

preschoolers who had lower reported fruit and total fruit and vegetable intakes. Since having a BMI in the overweight/obese range is a modifiable health risk factor, weight status of the mother may represent a target for intervention in effecting the fruit and vegetable varieties of preschoolers.

The significant bivariate relationship between breastfeeding and greater fruit variety, this result coincides with finding by Skinner et al (Skinner 2002). Skinner et al found that breastfeeding duration was a positive indicator of fruit variety in older children. Similarly, Skinner et al found no significant predictors of vegetable variety.

Research by Wardle et al and Cooke et al (Wardle 2007, Cooke 2004) which showed socioeconomic status to be related to fruit and vegetable variety, corroborates our bivariate analysis results which also showed maternal education and income to be significantly and independently associated with fruit and vegetable variety, though we found no relationship with income. One notable aberration from the bivariate trend seen in our research and elsewhere in literature, is research by Koui et al, which showed a surprising inverse relationship between mother's and father's education and mean daily vegetable consumption in Greek children (Koui 2008).

Prediction models of vegetable variety in this study yielded no significant predictor variables. The contrast between the predictive strength of the fruit variety models with the vegetable variety models supports prior research that suggests that there is a dichotomy between the treatment of fruit intake and vegetable intake (Zeinstra 2009). The findings that the same variables that predicted fruit variety, but not vegetable variety, also supports prior research which suggests that vegetable variety may be more difficult

to predict. The usual predictors of food intake do not seem to be related to vegetable intake in preschoolers.

Short-term weaning studies have shown that vegetable variety and consumption are lower for vegetables (Mennella 2008), and the findings of this analysis suggests that the challenge of promoting vegetable intake persists into the toddler years as well, despite breastfeeding exposure. As suggested by Mennella et al and shown by Zeinstra et al, children tend to rate vegetables the lowest in terms of how much they like the vegetables, while rating fruit/fruit juices and high-energy, dense foods as the most liked. Though researchers have suggested that this is a protective mechanism against intake of toxic plants, which are typically bitter, (Mennella 2008, Zeinstra 2009), Zeinstra et al also showed that, in comparing 4-5 year-olds with 7-8 year-olds and 11-12 year-olds, older age was a significant positive predictor of vegetable intake. These results suggest that, the palette for the flavors and textures of vegetables may require more time to develop.

The reported fruit and vegetable frequency results bear some similarities to the top 5 reported foods in the FITS study. Though the most frequently reported fruits and vegetables are similar in this group of preschoolers, the breastfed preschoolers had a higher percentage of variety coming from whole fruits and dark green/orange vegetables. These results suggest that, based on the counted fruit and vegetable varieties of the two-day intakes, the breastfed preschoolers may be consuming a more healthful diet.

Additionally, juice was the most frequently reported fruit across both infant feeding groups. This means that each fruit variety count likely includes one tally for 100% juice for 69 and 75% of BF and FF groups. Though juice was counted toward the

variety count in this study, prior studies have shown that the volume of juice consumption in toddlers can be problematic, adding excess calories and increasing weight in toddlers (O'Mara 2008, Pereira 2008).

Moreover, the significance of the mother's education and the preschooler's fruit variety and summed fruit and vegetable variety in predicting the preschooler's BMI z-score is a key finding that is somewhat supported by prior research. There has been little data specifically regarding preschoolers' fruit and vegetable intakes in relation to maternal characteristics. In one study of college students evaluating BMI and dietary variety, Brunt et al (Brunt 2008) found that students with the lowest BMI consumed a greater amount of vegetables, particularly leafy green vegetables. Kontogianni et al also found that adherence to the Mediterranean diet, a diet rich in fruits and vegetables, was a protective factor against overweight and obesity in Greek children and adolescents (Kontogianni 2010).

Limitations

The first limitation of this study is the cross sectional design, which is not adequate to fully predict outcomes of health behaviors (i.e.- choosing a mode of infant feeding and consuming/offering a variety of fruits and vegetables). Secondly, since the study population was uniquely made up of preschoolers born to overweight/obese women, the results may not be generalizable to preschoolers born to normal weight mothers. Additionally, this study used counts of the number of different fruits and vegetable that appeared in a two-day 24-hour dietary recall to assess variety. However, the measurement of variety did not include the amount of each fruit and vegetable

reported, just the occurrence in the preschooler's diet. Therefore, conclusions cannot be made drawn about the adequacy of the preschoolers' diet, since a healthful diet is comprised of both adequate volume and adequate variety (Krebs-Smith 1987, Ruel 2003).

The 24-hour dietary recall method has the inherent weakness of over/under-reporting by the mothers, in that, mothers may be less likely to report vegetables perceived as negative, such as fried potatoes (i.e.- french fries, potato tots, hashbrowns). In addition, mothers may be more likely to over-report fruits and vegetables perceived as positive, such as leafy green vegetables and cruciferous vegetables. Furthermore, inherent in this study design, the mother must act as a proxy for the preschooler's intake, since 2-3 year olds are not old enough to directly provide a recall of their 24-hour dietary intake. The mother acting as a proxy relies on the mother's memory what was served to the child and what was actually consumed by the child. If the child was away from the mother during the 24-hour period, this means that the mother must obtain the record of what was consumed from another party. This is seen with use of the Preschooler Daycare Intake form, which relies on the daycare provider to keep an accurate record of what the preschooler actually consumed while away from the mother.

Another weakness of the present study is the inability to control for timing of solid food introduction. Though the American Academy of Pediatrics recommends introducing solid foods at 6 months, the introduction of solid foods in the population tends to occur between 4 and 6 months. Preschoolers may have been subjected to novel flavors from solid foods given prior to four months of age. Lastly, mother's reported

breastfeeding history information retrospectively, thinking back to when the child was 0-12 months old. Since the mothers relied on memory to complete the breastfeeding history portion of the baseline questionnaire, errors could have been introduced. Though, research by Li et al concluded that maternal recall of infant feeding history is both valid and reliable in terms estimating breastfeeding initiation and duration for up to three years (Li 2005).

Conclusions

These results show that breastfeeding was related to fruit variety, but not vegetable variety. However, when controlling for other factors- infant feeding was no longer significant. Instead, maternal education and BMI were the significant predictors of fruit and vegetable intake. In addition, these results suggest that fruit and vegetable variety and maternal education are inversely related to child's BMI z-score at 2-3 years of age.

These results show that, while breastfeeding has many clear, proven benefits, full breastfeeding, in isolation, does not equate to increased fruit and vegetable variety in preschoolers of overweight and obese mothers. In reality, breastfeeding is a positive start, but is more easily overshadowed by additional factors such as mother's BMI and education. Therefore, these results suggest that, exposure to flavors transmitted in breastmilk within the first 3 months of life is not enough to impact later fruit and vegetable variety at 2-3 years of age.

Future Research

Based upon these conclusions, there are opportunities for further research into the determinants of vegetable intake, since this analysis identified no significant predictors of vegetable variety. In addition, the finding that mothers who had a higher BMI tended to have preschoolers with less varied intakes of fruits and vegetables makes investigation into the mothers' diets, focusing on fruit and vegetable intake habits, the next step for research.

CHAPTER IV

EPILOGUE

The collective findings of the present study and prior literature, seems to indicate that fruit variety is most strongly predicted by both maternal education and maternal BMI, while vegetable variety is not as clearly predicted by the same set of variables. In turn, fruit variety, total fruit and vegetable variety, and maternal education all emerged as significant negative predictors of child's BMI z-score. Therefore, it appears that fruit and vegetable variety, which bivariate results suggest can be positively influenced by breastfeeding over formula feeding, could be one mediator in the pathway of BMI status in a preschooler.

The primary problem encountered during the research process was the crudeness of the simple fruit and vegetable variety count. The variety count did not take into consideration the amount of the food item consumed which means that diet quality cannot be assessed by the present study. In addition, since a simple count of number of fruits and vegetables appearing in the diet is not readily used as a marker of disease/health risk, a more useful tool would have some compatibility in terms of health risk assessment- (i..e- quantifying the average amount of saturated fats consumed; ounces of lean protein/day).

To help combat this problem, the idea of fruit and vegetable variety should be further enlarged to the investigating total dietary variety with the added component of

dietary adequacy, as found in the Healthy Eating Index-2005 (HEI-2005). The HEI-2005 could then be related to the child's BMI status. By investigating total dietary quality and adequacy in terms of an index, the results can then be compared on a longitudinal basis. Additionally, the more refined dietary quality tool will enable researchers to assess preschooler adherence to the recommendations with the fruit and vegetable subgroups, in terms of variety and adequacy of total volume.

In addition, this research identifies overweight/obese mothers a potential interventional target audience for additional investigation. This study showed that the *most* overweight women also had preschoolers who had the lowest reported variety of fruits and vegetables. This means that mothers on the higher end of the spectrum may benefit the most from an intervention that addresses the mother's diet, which may translate to increased fruit and vegetable variety in their children. Additionally, overweight mothers who were also of a lower education level are also prime targets for intervention, since a higher education level of the mother was found to be a significant positive predictor of fruit variety in 2-3 year olds.

Lastly, the overarching goal of such research would be to stop the development of childhood obesity in these children who are at risk for overweight/obesity due to the overweight/obese status of their mother. Therefore, if fruit and vegetable variety is increased in the preschoolers, ideally, this would be concurrent with decreasing risk of overweight/obese in this group of preschoolers. This further supports the need for persistence in introducing foods and maintaining frequency of recurrence in the diet to help promote a varied diet of both fruits and vegetables.

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Figure 1. Theoretical Model

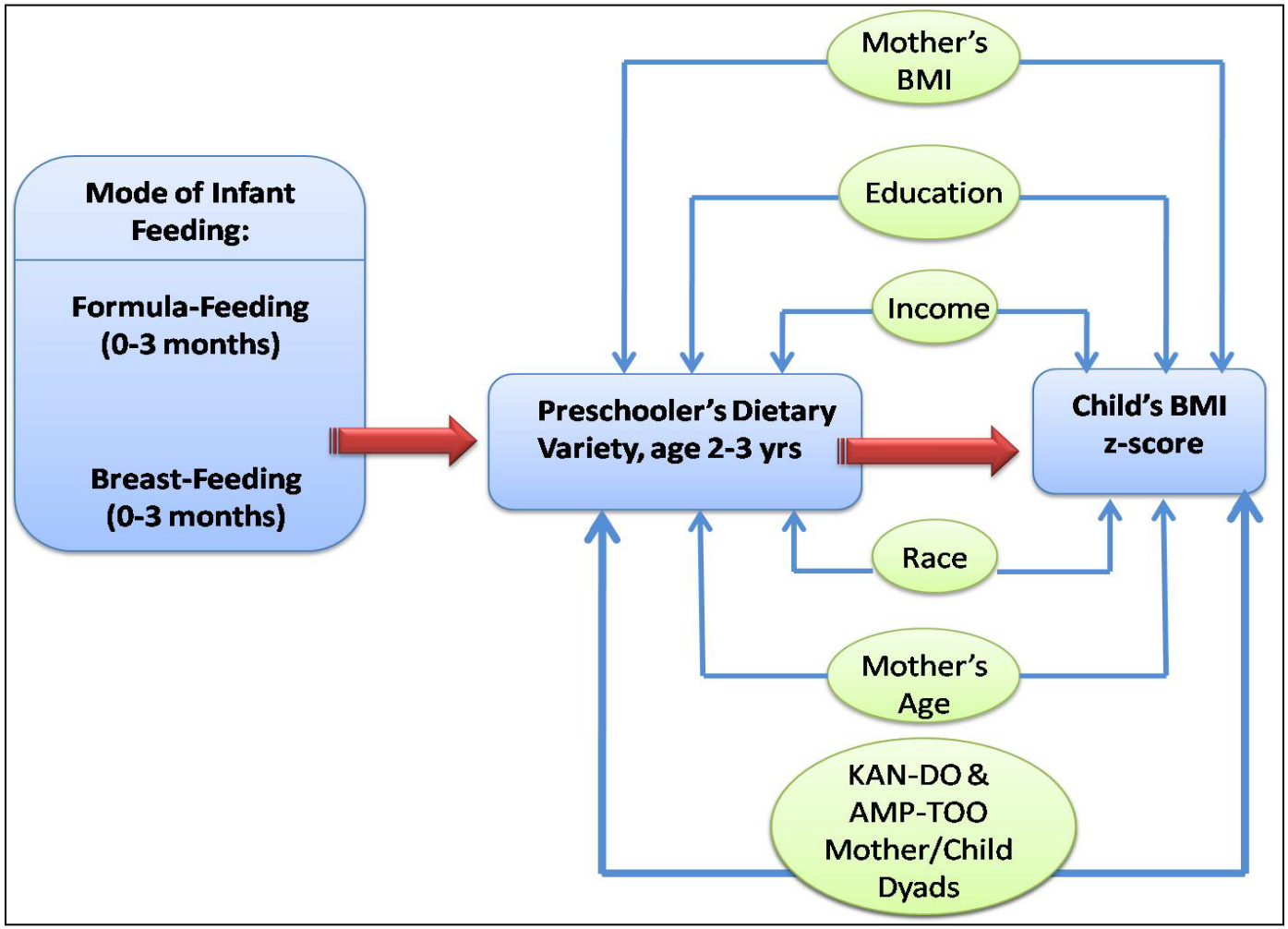

Figure 2. Sample Size Derivation

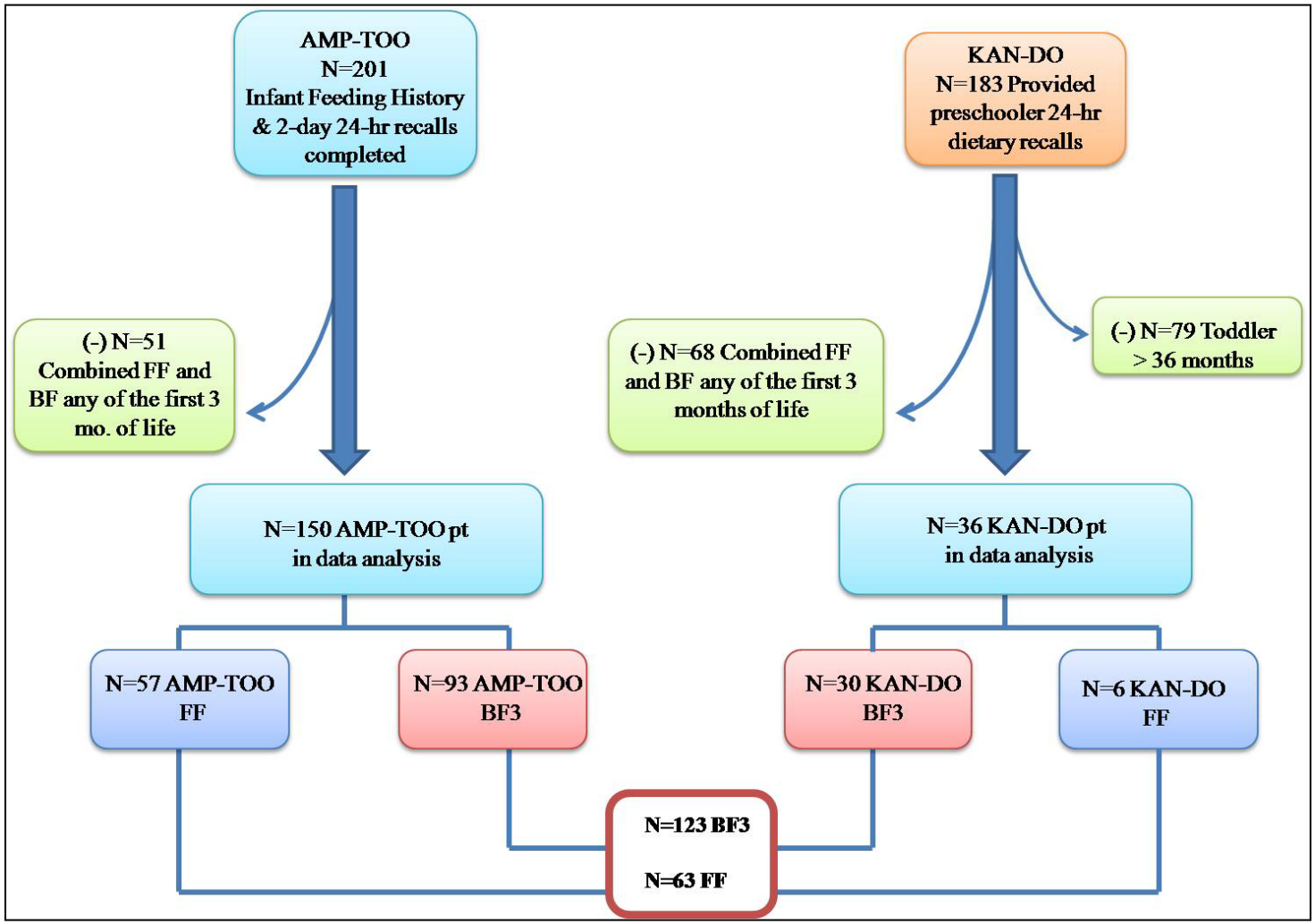

Table 1. Participant Characteristics

	Infant Feeding Group		
	Formula-Fed (FF) (n = 63)	**3-Months Fully Breastfed (BF) (n= 123)**	**P-value**
Child Characteristics			
Age (Months) mean (SD)	24.91 (2.92)	25.50 (2.92)	P = 0.21
Gender n (%)			P=0.94
Male	35 (55.56)	69 (56.10)	
Female	28 (44.44)	54 (43.90)	
BMI z-score-WHO (mean)(SD)	0.98 (1.08)	0.96 (0.09)	P=0.32
Maternal Characteristics			
Age mean (SD)	30.60 (6.01)	31.92 (4.67)	P=0.10
BMI mean (SD)	32.93 (6.28)	31.22 (4.83)	P=0.04*
Race (n) (%)			P=0.002*
White (1)	31 (48.21)	57 (75)	
Black & All Other (2)	32 (50.79)	19 (25)	
Education Grouping n (%)			P=0.0005*
Less than HS grad/HS grad (1)	17 (26.98)	8 (6.50)	
Vocat/Bus Sch/some college/Assoc (2)	16 (25.40)	46 (37.40)	
College Grad/Some Grad/Grad Educ (3)	30 (47.62)	69 (56.10)	
Income Grouping n (%)			P=0.21
$30,000 and under (1)	15 (23.81)	17 (13.93)	
S30,001 to $60,000 (2)	19 (30.16)	37 (30.33)	
$60,001 and higher (3)	29 (46.03)	68 (55.74)	
Food Characteristics			
Average Daily Servings mean (SD)			
Averge Fruit Servings/day	1.21 (0.98)	1.46 (1.22)	P=0.16
Average Vegetable Servings/day	0.82 (0.71)	0.76 (0.64)	P=0.60
Food Variety mean (SD)			
# fruits/2 days	2.70 (1.51)	3.25 (1.47)	P=0.02*
# vegetables/2 days	2.76 (1.61)	2.47 (1.54)	P=0.23
Total Fruit & Vegetable Variety/2 days	5.46 (2.36)	5.72 (2.26)	P=0.46

Table 2. Reported Fruit Variety & Frequencies of 2-day 24-Hour Dietary Recalls in Fully-Formula-Fed 2-3 Year-Olds, and 3-Months-Fully Breastfed 2-3 Year-Olds

Fully Formula-Fed: Fruits			**At Least 3 Months Fully Breastfed: Fruits**		
Fruits	**# Preschoolers Reporting Each Fruit**	**Percentage (N=63)**	**Fruits**	**# Preschoolers Reporting Each Fruit**	**Percentage (N=123)**
100% juice	47	74.60%	100% juice	85	69.11%
banana	28	44.44%	apples	51	41.46%
apples	13	20.63%	banana	50	40.65%
grapes	12	19.05%	grapes	30	24.39%
applesauce	10	15.87%	applesauce	26	21.14%
mixed fruit	9	14.29%	oranges	24	19.51%
raisins	9	14.29%	raisins	22	17.89%
oranges	8	12.70%	mixed fruit	16	13.01%
strawberries	7	11.11%	strawberries	15	12.20%
peaches	5	7.94%	peaches	13	10.57%
pears	5	7.94%	cantaloupe	13	10.57%
pineapple	4	6.35%	blueberries	12	9.76%
cantaloupe	3	4.76%	pineapple	8	6.50%
watermelon	3	4.76%	watermelon	8	6.50%
blueberries	2	3.17%	pears	5	4.07%
fruit leather	1	1.59%	cherries	5	4.07%
grapefruit	1	1.59%	cranberries	4	3.25%
plum	1	1.59%	raspberries	4	3.25%
			fruit leather	2	1.63%
			blackberries	2	1.63%
			honeydew	1	0.81%
			prunes	1	0.81%
			plum	1	0.81%
			mango	1	0.81%
			kiwi fruit	1	0.81%
			honeydew	1	0.81%
			nectarine	1	0.81%
			cranberries	1	0.81%
Fruit			**Fruit**		
Total	18		Total	28	
Whole*:	15	83.33%	Whole*:	25	89.29%
*(Excludes 100% juice, applesauce, & fruit leather)			*(Excludes 100% juice, applesauce, & fruit leather)		P < 0.01*

Table 3. Reported Vegetable Variety & Frequencies of 2-day 24-Hour Dietary Recalls in Fully-Formula-Fed 2-3 Year-Olds, and 3-Months-Fully Breastfed 2-3 Year-Olds

Fully Formula-Fed: Vegetables			**At Least 3-Months Fully Breastfed: Vegetables**		
Vegetables	**# Preschoolers Reporting Each Vegetable**	**Percentage (N=63)**	**Vegetables**	**# Preschoolers Reporting Each Vegetable**	**Percentage (N=123)**
green beans	28	44.44%	tomato sauce	36	29.27%
fried potatoes	21	33.33%	green beans	31	25.20%
tomato sauce	19	30.16%	fried potatoes	28	22.76%
corn	17	26.98%	carrots	24	19.51%
potatoes	15	23.81%	potatoes	24	19.51%
broccoli	13	20.63%	broccoli	21	17.07%
carrots	10	15.87%	corn	20	16.26%
mixed vegetables	8	12.70%	green peas	18	14.63%
green peas	6	9.52%	tomatoes	16	13.01%
mushrooms	3	4.76%	mixed vegetables	12	9.76%
cabbage	3	4.76%	iceburg lettuce	10	8.13%
greens	3	4.76%	spinach	9	7.32%
lima beans	2	3.17%	cucumber	6	4.88%
cucumber	2	3.17%	onions	6	4.88%
romaine lettuce	2	3.17%	cabbage	4	3.25%
tomatoes	2	3.17%	edamame	3	2.44%
peppers	2	3.17%	yellow squash	3	2.44%
onions	2	3.17%	green leaf lettuce	3	2.44%
sweet potatoes	2	3.17%	lima beans	3	2.44%
okra	1	1.59%	zucchini	3	2.44%
iceburg lettuce	1	1.59%	greens	2	1.63%
celery	1	1.59%	sweet potatoes	2	1.63%
wax beans	1	1.59%	peppers	2	1.63%
edamame	1	1.59%	celery	2	1.63%
zucchini	1	1.59%	mushrooms	1	0.81%
			snow peas	1	0.81%
			asparagus	1	0.81%
			pumpkin	1	0.81%
			avocado	1	0.81%
Vegetables			**Vegetables**		
Total	25		Total	30	
Dark Green, & Orange	6	24%	Dark Green, & Orange	9	30% P = 0.01*

Table 4. Generalized Linear Model: Predictors of Variety of Fruit Intake in Fully-Formula-Fed and 3-Months-Fully Breastfed Preschoolers

2-day Fruit Variety Predictors		
Variable	**Overall Effect Size**	**Overall P-value**
Intercept (Overall)	1.04	0.01
Education		
Reference: ≤ HS Grad	0.00	
Some college/Assoc degree	0.33	0.06
≥ College degree	0.36	0.04*
Income		
Reference: ≤ $30,000/year	0.00	
$30,001 - $60,000/year	-0.02	0.89
> $60,000/year	0.04	0.82
Race		
Reference: White	0.00	
Black & All Other	-0.06	0.57
Mother's Age		
Reference: Avg = 31.27 yrs	0.01	0.15
Mother's BMI		
Reference: Avg = 32.08	-0.02	0.02*
Study Classification		
Reference: AMP-TOO		
KAN-DO	-0.20	0.19
Infant Feeding Group		
Reference: Formula (FF)	0.00	
At Least 3 Months BF (BF)	0.09	0.37

Table 5. Generalized Linear Model: Predictors of Variety of Vegetable Intake in Fully-Formula-Fed and 3-Months-Fully Breastfed Preschoolers

2-day Vegatable Variety Predictors		
Variable	**Overall Effect Size**	**Overall P-value**
Intercept (Overall)	0.97	0.02
Education		
Reference: ≤ HS Grad	0.00	
Some college/Assoc degree	0.18	0.29
≥ College degree	0.13	0.43
Income		
Reference: ≤ $30,000/year	0.00	
$30,001 - $60,000/year	-0.01	0.94
> $60,000/year	-0.13	0.40
Race		
Reference: White	0.00	
Black & All Other	0.13	0.26
Mother's Age		
Reference: Avg = 31.27 yrs	0.004	0.66
Mother's BMI		
Reference: Avg = 32.08	-0.006	0.53
Study Classification		
Reference: AMP-TOO	0.00	
KAN-DO	-0.18	0.25
Infant Feeding Group		
Reference: Formula (FF)	0.00	
At Least 3 Months BF (BF)	-0.08	0.41

Table 6. Generalized Linear Model: Predictors of Variety of Total Fruit and Vegetable Intake in Fully-Formula-Fed and 3-Months-Fully Breastfed Preschoolers

2-day Total Fruit & Vegetable Variety Predictors		
Variable	**Overall Effect Size**	**Overall P-value**
Intercept (Overall)	1.72	<0.001
Education		
Reference: ≤ HS Grad	0.00	
Some college/Assoc degree	0.24	0.04*
≥ College degree	0.24	0.05
Income		
Reference: ≤ $30,000/year	0.00	
$30,001 - $60,000/year	-0.02	0.85
> $60,000/year	-0.04	0.70
Race		
Reference: White	0.00	
Black & All Other	0.03	0.71
Mother's Age		
Reference: Avg = 31.27 yrs	0.009	0.19
Mother's BMI		
Reference: Avg = 32.08	-0.01	0.03*
Study Classification		
Reference: AMP-TOO	0.00	
KAN-DO	-0.19	0.08
Infant Feeding Group		
Reference: Formula (FF)	0.00	
At Least 3 Months BF (BF)	0.09	0.37

Table 7. Generalized Linear Model: Predictors of Child's BMI Z-score

Child's BMI Z-score Predictors		
Variable	**Overall Effect Size**	**Overall P-value**
Intercept (Overall)	1.83	0.006
Education		
Reference: ≤ HS Grad	0.00	
Some college/Assoc degree	-0.67	0.01*
≥ College degree	-0.30	0.27
Income		
Reference: ≤ $30,000/year	0.00	
$30,001 - $60,000/year	0.08	0.74
> $60,000/year	0.08	0.74
Race		
Reference: White	0.00	
Black & All Other	0.20	0.97
Mother's Age		
Reference: Avg = 31.27 yrs	0.00	0.10
Mother's BMI		
Reference: Avg = 32.08	-0.01	0.68
Study Classification		
Reference: AMP-TOO	0.00	
KAN-DO	-0.19	0.75
Infant Feeding Group		
Reference: Formula (FF)	0.00	
At Least 3 Months BF (BF)	0.05	0.75
2-day Total F&V Variety	-0.07	0.04*

Appendix A: Recruitment Postcard

Appendix B. KAN-DO Consent Form

Form M0345

DUKE UNIVERSITY HEALTH SYSTEM

Consent To Participate In A Research Study
KAN-DO: A Family-Based Intervention to Prevent Childhood Obesity

Introduction

The Duke University Medical Center (DUMC) and The University of North Carolina at Greensboro (UNCG) are conducting a research study about the importance of preventing children from becoming overweight. As a woman who recently delivered a baby and is the parent of a child between 2 and 5, you and your preschooler are eligible to participate in this study. This study is being sponsored by a grant from the National Institutes of Health (NIH). Portions of Dr. Østbye's, Dr. Lovelady's and the research team's salaries are being paid by this grant.

Research studies include only people who choose to take part. Please read this consent form carefully and take your time making your decision. As your study staff discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand and answer any questions you may have. We encourage you to talk with your family and friends before you decide to take part in this research study. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

Purpose of the Study

The goal of the study is to determine whether the KAN-DO intervention can encourage healthy eating, increased physical activity, and healthy weights among participating women and their children.

How the Study Works

Women who recently had a baby in the Raleigh/Durham/Chapel Hill or Greensboro/Winston-Salem/High Point regions of North Carolina, and also have a child aged 2-5, are English speaking, and are age 18 or older will be eligible to participate. A total of 400 women and 400 children will be enrolled in the study. The study will take place over the course of about 2 years.

If you agree to be in the study, we will ask you and your child to visit us at Duke (for the Triangle families) or UNCG for the Triad families). We will have you sign this consent form and we will give you a copy for your records. At that visit, we will weigh and measure you and your child. We will measure your waist and hip circumference. You will be asked to complete a 30-minute paper and pencil survey about your family's health and parenting behaviors. When you and your child attend a one-hour session at either DUMC or UNCG, you will be fitted for an activity monitor that you will both wear for one week, and then return via pre-paid envelope or in person. After your visit, you will be called by The University of North Carolina – Greensboro for a detailed 20-30 minute survey about your diet on two separate days within a two-week period. Immediately following that survey, you may also agree to do another detailed 20-30 minute survey about your child's diet on two separate day (this is optional and you can decide today if you would like to do this). After completing the phone dietary survey(s), you will be randomly assigned to one of two groups using a process like the flip of a coin.

If you are assigned to **Group 1,** you will receive a monthly newsletter about boosting reading skills in your child. You will complete another set of assessments, including a one hour office visit, paper survey, phone dietary survey, weigh-in, and activity monitor, about 10 months from now. You will be contacted again after another year (about 2 years from now) for a shorter paper survey and weigh-in only.

If you are assigned to **Group 2**, you will also complete another set of assessments (including a one hour office visit, paper survey, phone dietary survey (or surveys if you decide to complete a phone surveys about your preschooler's diet), weigh-in, and activity monitor) in about 10 months. You will be contacted again after another year (about 2 years from now) for a shorter paper survey and weigh-in only. In addition, as part of **Group 2,** you would be asked to do the following:

1. receive a family kit in the mail once a month designed to help you prevent your child's chance of becoming overweight; the kit includes information and activities about healthy eating, physical activity, and parenting strategies. The kit's activities will take about an hour to complete each month. We encourage healthy behaviors. However, we will discourage the use of dietary supplements such as pills, herbal preparations, or other supplements unless prescribed by your doctor. There is very little information available on the safety and effectiveness of dietary supplements, and, in some cases, they can be dangerous;
2. participate in one 90 minute group classes emphasizing physical activity, healthy eating, and parenting strategies, and;
3. receive eight phone calls from a wellness coach during the study period to talk about how your family might incorporate the parenting skills and lifestyle changes that are part of the KAN-DO program. These phone calls, each about 20 minutes, will be audio recorded for quality assurance. Digital files of these recordings are saved on a password protected external hard drive, used only by counseling staff, and will be destroyed 6 years after the study has ended.

Please initial one of the following statements:

_____ I agree to take part in the phone surveys regarding my preschooler's diet (two phone surveys in the next few weeks, and another two phone surveys in about 9 months)

_____ I do not agree to take part in the phone surveys regarding my preschooler's diet.

Benefits of participation

Expected benefits to Group 1 participants may include increased bonding between the mothers and preschoolers due to the reading intervention. Expected benefits to Group 2 participants may include increased health and well-being, achieving and maintaining a healthy weight in the children and weight loss for the mothers, and an increase in physical activity and improved nutrition and dietary habits. The indirect benefit of the study is a better general understanding of successful interventions to increase weight loss and healthy weight-related behaviors in mothers and their children.

Risks or discomforts

When promoting increased physical activity, there is a small risk of injury or other adverse (bad) events. We have taken precautions to minimize these risks, however, there may be unforeseen problems that we have not anticipated. In case of injury during physical activity that is directly related to this study, please inform us immediately by calling the Duke study coordinator at 1-866-681-0860 or the UNCG study coordinator at 1-866-99-KAN-DO and describing the event, and what actions you have taken as a result (for instance, if you called a doctor).

Immediate necessary medical care is available at Duke University Medical Center in the event that you are injured as a result of your participation in this research study. However, there is no commitment by Duke University, Duke University Health System, Inc., or your Duke physicians to provide monetary compensation or free medical care to you in the event of a study-related injury. Further information concerning this or your rights as a research subject may be obtained from the Duke University Health System Institutional Review Board (IRB) Office at (919) 668-5111. For Triad participants, contact the UNCG Office of Research Compliance at (336) 256-1482.

Remuneration and/or expense reimbursements

As part of this study, if you are assigned to Group 2, you will be asked to participate in one group classes about healthy habits in the home. You will not be charged fees for these activities and food will be provided. In addition, the free kits mailed to families in Group 2 include information and activities for adults and children. Families may receive prizes for completing activities in the family kits.

All women, regardless of group assignment, will receive a total of $100 for completing all of the assessments ($30 for the first set, $30 for the second and $40 for the third). Free parking is available whenever visits to the study site are required. Mileage reimbursement is available to families living more than 20 miles away at the current federal mileage reimbursement rate.

If you agree to take part in the optional surveys about your preschooler's diet, you will be paid an additional $10 for the first set of assessments, and $10 for the second set of assessments.

Additional costs to participate

Transportation to and from study activities will be the responsibility of the participant. The sponsor of the study, the National Institutes of Health, is providing the newsletter, classes, mailed materials and wellness coach consultations to women free of charge.

Participation

You and your child may choose not to be in the study, or, if you and your child agree to be in the study, you or your child may withdraw from the study at any time. If you withdraw from the study, no new data about you or your child will be collected for study purposes unless the data concern an adverse event (a bad effect) related to the study. If such an adverse event occurs, we may need to review your or

your child's medical record. All data that have already been collected for study purposes, and any new information about an adverse event related to the study, will be sent to the study sponsor.

Your decision not to participate or to withdraw yourself or your child from the study will not involve any penalty or loss of benefits to which you are entitled, and will not affect your access to health care at Duke or anywhere else. If you do decide to withdraw yourself or your child, we ask that you contact Dr. Østbye in writing and let him know that you are withdrawing from the study. His mailing address is DUMC Box 2914, Durham, NC 27710.

Confidentiality

As part of this study, you will be asked questions about your weight and weight history, your eating patterns and habits, and your level of physical activity, in surveys conducted by telephone and in-person. We will also weigh you and your child three times. Data stored electronically will be in files and computers protected by password access. Any hardcopy records will be kept in locked metal filing cabinets at the study sites.

Study records that identify you or your child will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you or your child will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS) or The University of North Carolina – Greensboro (UNCG). For records disclosed outside of DUHS or UNCG, you and your child will be assigned a unique code number. The key to the code will be kept in a locked file in Dr. Østbye's office. If you are randomly selected for Group 2, audio-recordings of your phone calls with the wellness coach will be stored electronically on a password protected external hard drive.

If the researchers have reason to believe a child is being abused (or has been abused), study staff are required by North Carolina state law to file a report with the appropriate agencies. If the researcher has reason to believe you may be a harm to yourself or others, our study physician will contact you for appropriate follow-up. In addition, your records may be reviewed in order to meet federal or state regulations. Reviewers may include, for example, representatives from the National Institutes of Health, the Duke University Health System Institutional Review Board or the University of North Carolina-Greensboro Office of Research Compliance. If any of these groups review your research record, they may also need to review your or your child's research record.

The study results will be retained in your research record for six years after the study is completed At that time, information identifying you or your child will be removed from such study results at DUHS or UNCG. This information may be further disclosed by the sponsor of this study, the National Institutes of Health. If disclosed by the sponsor, the information is no longer covered by the federal privacy regulations

DUKE UNIVERSITY HEALTH SYSTEM

Consent To Participate In A Research Study
KAN-DO: A Family-Based Intervention to Prevent Childhood Obesity

The study results will be retained in your child's research record for six years after the study is completed or until your child reaches the age of 21, whichever is longer. At that time either the research information not already in your child's medical record will be destroyed or information identifying your child will be removed from such study results at DUHS. Any research information in your child's medical record will be kept indefinitely.

Dr. Cheryl Lovelady and her staff from the Nutrition Department at UNCG will conduct two interviews regarding your food intake, one in the next two weeks, and another in approximately 10 months. UNCG will have access to some information about you (your name and telephone number) so that they can contact you. UNCG will be conducting the surveys on Duke's behalf and acting as agents of Duke, and therefore are held under the same confidentiality standards as Dr. Østbye's study team.

Questions

Immediate necessary medical care is available at Duke University Medical Center in the event that you are injured as a result of your participation in this research study. However, there is no commitment by Duke University, Duke University Health System, Inc., or your Duke physicians to provide monetary compensation or free medical care to you in the event of a study-related injury. For questions about the study or research-related injury, contact Dr. Truls Østbye at (919) 661-0331 or Dr. Cheryl Lovelady at (336) 256-0310 during regular business hours.

For questions about your rights as a research participant, Triangle participants should contact the Duke University Health System Institutional Review Board (IRB) Office at (919) 668-5111. Triad participants should contact Eric Allen in the UNC-Greensboro Office of Research Compliance at (336) 256-1482.

STATEMENT OF CONSENT

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told that I may contact the Duke University Health System Institutional Review Board (IRB) Office at (919) 668-5111 if I have questions about my rights as a research subject, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I have read this consent form and agree to be in this study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed copy of this consent form."

_____ _____
Signature of Subject Date

_____ _____
Signature of Person Obtaining Consent Date

Appendix C. AMP-TOO for Twos! Consent Form

DUKE UNIVERSITY HEALTH SYSTEM

Family Factors Influencing Maternal Weight Change Postpartum
Truls Østbye, MD, MPH, PhD and Bernard Fuemmeler, PhD, MPH
IRB Registry number: **8939-07-9ER**
MOTHER CONSENT

Introduction
The Duke University Medical Center is conducting a research study about the importance of families in weight-loss and weight-related behaviors. As a woman with a young child, you are eligible to participate in this study. This study is being sponsored by a grant from the National Institutes of Health. Portions of Drs. Fuemmeler's and Østbye's and the research team's salaries are being paid by this grant.

Research studies include only people who choose to take part. Please read this consent form carefully and take your time making your decision. As your study staff discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below.

Purpose of the Study
The goal of the study is to determine whether similarities and differences among family members' (i.e., children and partners') health-related behaviors affect weight loss, calorie intake, and level of physical activity among women in the Active Mothers Postpartum (AMP) study.

How the Study Works
The study will take place over two weeks. First, women and their two-year-old children will come to the AMP office have their weight and height measured. At that visit, women will complete a pen-and-paper survey about eating and physical activity behaviors and attitudes in their families. They will be asked to take home a self-addressed stamped envelope containing information about the study and a survey packet with for their partners to complete. Then, within the next two weeks, they will be asked to 1) complete a detailed phone survey about their child's dietary intake, and 2) return the partner's survey. A total of 450 families will be enrolled.

Benefits of participation
You will receive no direct benefit from participating in this study. The indirect benefit of the study is to better understand how families can influence interventions to increase weight loss and healthy weight-related behaviors.

Risks or discomforts
There should be no risks to you, your child or your partner as a result of this study. There is, however, the potential risk of loss of confidentiality. Every effort will be made to keep your information confidential, however, this can not be guaranteed. You may feel uncomfortable answering some of the questions. You may refuse to answer any of the questions and you may take a break at any time during the study. You may stop your participation in this study at any time.

Remuneration and/or expense reimbursements
You will receive money compensation for your time spent completing the surveys/weigh-ins. You will receive $15 for completing the phone survey and child weigh-in, and an additional $10 for your partner's survey.

Participation

You or your partner may choose not to be in the study, or, if you agree to be in the study, you may withdraw from the study at any time. Also, you may participate and complete your part of the study even if your partner does not choose to participate. If you withdraw from the study, no new data about you will be collected for study purposes. In the process of our interview, should we become concerned about any developmental issues of your child, we will ask you to seek further evaluation by your family care or pediatric provider.

Your decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits to which you are entitled, and will not affect your access to health care at Duke or your participation in AMP. If you do decide to withdraw, we ask that you contact Drs. Fuemmeler or Østbye in writing and let him know that you are withdrawing from the study. His mailing address is DUMC Box 2914, Durham, NC 27710.

Confidentiality

Any data stored electronically will be in files and computers protected by password access. Any hardcopy records will be kept in locked metal filing cabinets at the study sites. Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of Duke University Health System (DUHS). For records disclosed outside of DUHS, you will be assigned a unique code number. The key to the code will be kept in a locked file in Dr. Fuemmeler's office.

In addition, your records may be reviewed in order to meet federal or state regulations. Reviewers may include, for example, representatives from the National Institutes of Health, or the Duke University Health System Institutional Review Board.

The study results will be retained in your research record for at least six years after the study is completed. At that time either the research information will be destroyed or information identifying you will be removed from such study results at DUHS. This information may be further disclosed by the sponsor of this study, the National Institutes of Health. If disclosed by the sponsor, the information is no longer covered by the federal privacy regulations

Dr. Cheryl Lovelady or her staff from the Nutrition Department of the University of North Carolina at Greensboro (UNC-G) will conduct two telephone interviews regarding your child's diet. UNC-G will have access to some information about you (your name and telephone number) so that they can contact you. UNC-G will be conducting the surveys on Duke's behalf and acting as an agent of Duke, and therefore be held under the same confidentiality standards as Dr. Østbye's study team.

Questions

For questions about the study or a research related injury, contact either Dr. Bernard Fuemmeler at (919) 681-7171, or the study coordinator, Margaret Pendzich, at (919) 668-3962 during regular business hours. For questions about your rights as a research participant, contact the Duke University Health System Institutional Review Board (IRB) Office at (919) 668-5111.

Authorization

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to be in this study, with the understanding that I may withdraw at any time. I have been told that I will be given a signed copy of this consent form."

Signature of subject:_____ Date: _____

Signature of person obtaining consent: _____ Date: _____

Appendix D. Preschooler Daycare Intake Record

Food & Beverage Intake for _____

Date _____

Please include the time that the child ate or drank and what type of meal it was: breakfast, lunch, snack, dinner, etc.

List the foods and beverages that the child had, along with the exact amount that the child consumed. Specify brand names if they are available.

Remember, we only need to know what the child actually ate or drank -- not what he/she was served.

MOM -- Please review this after completed by the daycare to make sure that amounts and brand names if available are provided.

Time_____ **Meal** _____

Food or Beverage **Amount eaten or drank**

_____

_____

_____

_____

_____

Time_____ **Meal** _____

Food or Beverage **Amount eaten or drank**

_____

_____

_____

_____

_____

_____

Time_____ Meal _____

Food or Beverage Amount eaten or drank

_____

_____

_____

_____

_____

Time_____ Meal _____

Food or Beverage Amount eaten or drank

_____

_____

_____

_____

_____

Time_____ Meal _____

Food or Beverage Amount eaten or drank

_____

_____

_____

_____

_____

If you have questions, please contact:
KAN-DO Study Office
kando@mc.duke.edu
Triangle site: 1-877-WE-KANDO • Triad site: 1-866-99-KANDO or 334-9842